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Effect of green manure *Sesbania sesban* and nitrification inhibitor encapsulated calcium carbide (ECC) on soil mineral-N, enzyme activity and nitrifying organisms in a rice–wheat cropping system

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Abstract

Green manure *Sesbania sesban* (*S. sesban*) and the nitrification inhibitor encapsulated calcium carbide (ECC) have been used to improve N supply and management in rice–wheat production systems in India. However, the ecological impact of combined use of these materials is largely unknown. We conducted a net-house pot culture experiment for 2 years, to investigate the effects of *S. sesban* and ECC on mineral N availability (NH_4^+ and NO_3^-), soil enzyme activities (dehydrogenase and nitrate reductase) and populations (MPN) of nitrifying organisms under a rice–wheat cropping system. Green manure *S. sesban* and ECC (+ECC or –ECC) were applied along with urea in various combinations to hybrid rice under flooded conditions. For wheat, it was urea alone or urea + ECC. Soil samples were studied at 10 days after top dressing, i.e. 40 days after rice transplanting and 35 days after wheat sowing, for above characteristics. The mineral-N in soil revealed the significant effect of combined use of *S. sesban* and ECC to enhance NH_4^+ and total mineral-N ($NH_4^+ + NO_3^-$) contents. Dehydrogenase and nitrate reductase activities and population (MPN) of ammonia oxidizing bacteria (AOB) revealed a significant reduction in soils, whereas nitrite oxidizing bacteria (NOB) remained almost unaffected ($P > 0.05$) in response to application of ECC with *S. sesban* and urea. Our results suggest that slow release of acetylene (C_2H_2) from ECC has reduced ammonia mono-oxygenase with reducing population of AOB, and has the potential to retard the enzyme activities in favor of C and N conservations in a semi-arid agro-ecosystem.

Keywords: Encapsulated calcium carbide; Green manure; Nitrification inhibitor; Nitrifying organisms; Rice; Soil enzymes; Wheat

1. Introduction

Rice–wheat production systems occupy 24 million ha of cultivated land in the Asian subtropics [23]. About half of the total area is in north-eastern India [22] and represents a major cropping system for sustaining food security in the country. Because of the importance, this system consumes most of the N fertilizer used in the region. Further, to ensure India's food grain production to meet the ever-increasing demands, cultivars of hybrid rice have been introduced in recent years. Such cultivars have shown 20–30% higher yields but require more N than the existing high yielding varieties. However, increasing fertilizer cost and concern for sus-

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tainable soil quality and ecological stability in relation to chemical fertilizer use have emerged as important issues [3]. As a result, there has been much effort to enhance application of locally available organic resources of plant nutrients and to adopt strategies (e.g. nitrification inhibitors), which have potential to supplement and/or conserve N, enhance efficiency and reduce losses.

For rice in India during the monsoon or wet season (July–October), green manuring is practiced 15–30 days before transplanting to allow the decomposition and release of nutrients. Patterns of decomposition and nutrient release from green manures and their benefits to improve nutrient availability in soil and crop uptake have been studied in the past [38,39]. The green manure, *Sesbania sesban*, is a fast growing leguminous shrub, which decomposes rapidly when incorporated in soil [29,39]. It may, therefore, be grown simultaneously on spare or marginal lands or field bunds during rice growth and serve as a N source (at least partly) for top dressing of rice [31]. Since top dressing of N constitutes almost 50% of the total N application to rice and wheat in the region, efficient use of this N is also very important. However, information on the transformations and benefits of top dressed green manures to rice yields, particularly in hybrid rice, are limited.

Application of nitrification inhibitor, such as nitrapyrine or acetylene, with urea-based fertilizers is another promising approach to improve N management in agriculture. Interest in the use of nitrification inhibitors stems from the fact that these materials inhibit or rather retard nitrification process in the soil and reduces the chances of N loss by various mechanisms and thereby enhances N uses by crops. Because of the coating of calcium carbide (CaC_2) encapsulated calcium carbide (ECC) releases acetylene (C_2H_2) in soil for a long time, thereby delaying the nitrification process in the soil [4,5,15]. Although considerable work has been done in the past with ECC for enhancing nitrogen use by different crops [15], no information is available where green manure and nitrification inhibitor (such as ECC) are used together for enhancing the use efficiency of applied N by crops, and assessing their ecological impacts on soil enzyme activities and functional microbial communities, such as nitrifying organisms, which are driving forces for N cycling and sensitive indicators of soil quality [21].

The purpose of the present experiment was to find out the effect of application of ECC (a nitrification inhibitor) with urea (a major N fertilizer in India) and *S. sesban* (a green manure) on mineral N availability, soil enzyme activities and population of nitrifying organisms involved in N cycling under the rice–wheat production system of Northern India.

2. Materials and methods

2.1. Experimental site and treatments

This investigation was carried out during 2 years (July 1999 to April 2001) in a net-house under natural environmental conditions, as prevailed in field situation, at the Indian Agricultural Research Institute (IARI), New Delhi (longitude 77°12′E, latitude 28°38′ N, and altitude 239 m above mean sea level). The climate of the region is subtropical, semi-arid. The average annual rainfall is 670 mm, about 80% of which occurs from June to September. The mean maximum and minimum temperatures during the growth of rice (July–October) are 35 and 18 °C; while during wheat growth (November–April) they are 22.6 and 6.7 °C, respectively. Bulk soil, loamy in nature (*Typic ustochrept*, FAO: Cambisol), from 0 to 15 cm depth was collected (June 1999) from the research farm of the institute (IARI), gently ground to a 6-mm sieve and 4 kg placed in glazed porcelain pots. The physico-chemical analysis [28] of the soil showed initial pH (1:2 w/v water) 8.2, electrical conductivity (1:2 w/v water) 0.53 dS m^{-1}, CEC 8.5 $cmol_c$ kg^{-1}, total organic-C 4.4 g kg^{-1}, total-N 0.52 g kg^{-1}, Olsen-P 0.009 g kg^{-1}, and ammonium acetate extractable K 0.15 g kg^{-1}. Rice was grown in 1999 and 2000 during the wet or monsoon season of India (July–October). Treatments were the same in both years but soil measurements were only made in the second year.

The amount of N to rice applied through urea or urea + *Sesbania* was 107 mg kg^{-1}, and ECC was added at 10% of N applied (Table 1 for treatment details). Before transplanting of rice (*cv* PRH 10) in puddled soil [11], fertilizer grade single super phosphate, and muriate of potash were also added to supply the equivalent of 11.6 mg P kg^{-1} and 22 mg K kg^{-1}, respectively. *Sesbania* (as a green manure) chosen in this study for top dressing contained on average 3.4% N. It was grown on the institute farm, collected after 20 days growth, chopped (tender stems 1–3 cm) and mixed in soil together with urea. During rice growth (July–October 1999 and 2000), the incidence of disease or pests was regularly examined and taken care of. Also any weeds in the pots were removed by hand. Irrigation to the pots was given using tap water to maintain flooded condition (5 cm depth) until 15 days before harvesting.

After harvest of above ground biomass (grain and straw) of rice in October, the soils in pots were allowed to dry until wheat was sown in the following November (in both the years 1999 and 2000). A small trowel was used to loosen the soil, break the aggregates, and mix the litters and stubbles of previous rice in the pots. Finally, the soil surface of each pot was leveled by hand to give a good seedbed. For fertilizer application, 27 mg N kg^{-1} soil was applied as urea just before the sowing of wheat, either with or without ECC. The remaining 27 mg N kg^{-1} was applied as urea (+ECC or –ECC) 25 days after sowing (Table 1 for treatment details). Other nutrients (P and K as described for rice) were applied uniformly at the time of sowing along with the urea. Wheat seed (*cv* UP 2338), pre-soaked in water overnight, was sown at 1.5 cm depth in all pots. Any weeds in the pots were removed by hand. Irrigation to the pots was given in equal amounts using tap water.

2.2. Soil sampling and analyses

Soil samples were collected on the 10th day after top dressing in the second year of the experiment, i.e. 40 days after rice transplanting and 35 days after wheat sowing. Two soil cores (0–15 cm depth) were collected from each replication (pot) non-destructively using a narrow tube (2 cm dia), and pooled for analyses. A total of about 100 g soil from each pot was obtained for various analyses. Except mineral N, all analyses were performed on stored samples (4 °C).

For mineral N (NH_4^+ and NO_3^-) contents, soil was extracted in the next day after sampling using 2 M KCl and analyzed following the procedures as described by Keeney and Nelson [18]. Dehydrogenase activity was determined by monitoring the rate of production of tri-phenyl formazon (TPF) from tri-phenyl tetrazolium chloride (TTC), using the method of Klein et al. [20]. For the assay of nitrate reductase activities, 5 ml of 0.1 M KNO_3 solution was added to 5 g soil, incubated at 28 °C for 24 h and the amount of NO_2^- formed was estimated [34]. Most probable number (MPN) of ammonia and nitrite oxidizing bacteria (NOB) were enumerated using the media of Schmidt and Belser [35] with 10-fold dilutions from 10^{-3} to 10^{-7} and incubation at 28 °C for 10 weeks. The presence of ammonia oxidizers was revealed by the presence of NO_2^- and/or NO_3^- using Morgan's reagent. The presence of nitrite oxidizers was revealed by the absence of nitrite using Griess–Ilosway's reagent. The MPN of nitrifying microorganisms was estimated by Cochran's [10] method.

2.3. Statistical analyses

The whole experiment was conducted following a completely randomized design (CRD) with four replications. One-way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) for comparison of means were performed using SPSS 10 window version. Unless otherwise stated, the level of significance referred to in the results is $P < 0.05$.

3. Results

3.1. Mineral N

The data on soil mineral N (NH_4^+ and NO_3^--N) at 10 days after topdressing (i.e. 40 days after transplanting) of rice revealed significant ($P < 0.001$) differences among treatments (Table 2 and Fig. 1). There was significantly higher NH_4^+ or NO_3^--N concentrations due to application of ECC with urea and *S. sesban* (S3 and S4, Table 1) than in the absence of ECC. Total mineral N contents in soil were also significantly higher ($P < 0.05$ according to DMRT) in these treatments (S3 and S4) than others (S0, S1, S2).

Soils under the wheat crop, however, had significantly higher (Table 2 and Fig. 1) NO_3^--N (28 mg kg^{-1}) concentrations in urea treated soil (S1)

Table 1
Treatments for rice and wheat crops in 1999/2000 and 2000/2001

Treatment	Rice (July–October)	Wheat (November–March)
S0	Control (i.e. 0 N)	Control (i.e. 0 N)
S1	Urea N 50% at transplanting and 50% after 1 month	Urea N 50% at sowing and 50% after 25 days
S2	Urea N 50% at transplanting and urea N 25% + *S. sesban* N 25% after 1 month	Urea N 50% at sowing and urea N 50% after 25 days
S3	Urea N 50% (+ECC) at transplanting and urea N 25% + *S. sesban* N 25% after 1 month	Urea N 50% (+ECC) at sowing and urea N 50% after 25 days
S4	Urea N 50% (+ECC) at transplanting and urea N 25% + *S. sesban* N 25% (+ECC) after 1 month.	Urea N 50% (+ECC) at sowing and urea N 50% (+ECC) after 25 days

Total N applied for rice was at 107 mg kg^{-1} and for wheat 54 mg kg^{-1} soil; ECC: encapsulated calcium carbide.

Table 2
Effect of treatments on total mineral N (NH_4^+-N and NO_3^--N), enzyme activities and density of nitrifying organisms in soils under rice and wheat crops. The results are based on one measurement at 10 days after the treatment (top dressing)

Source of variation	NH_4^+-N	NO_3^--N	TMN	Dehydrogenase	Nitrate reductase	AOB	NOB
Treatments (rice)	< 0.001	0.001	< 0.001	< 0.001	< 0.001	0.05	NS
Treatments (wheat)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.04	NS

Data are *P* values of the one-way ANOVA; NS: not significantly different ($P > 0.05$); TMN: total mineral N, i.e. $(NH_4^+ + NO_3^-)$-N; AOB: ammonia oxidizing bacteria; NOB: nitrite oxidizing bacteria.

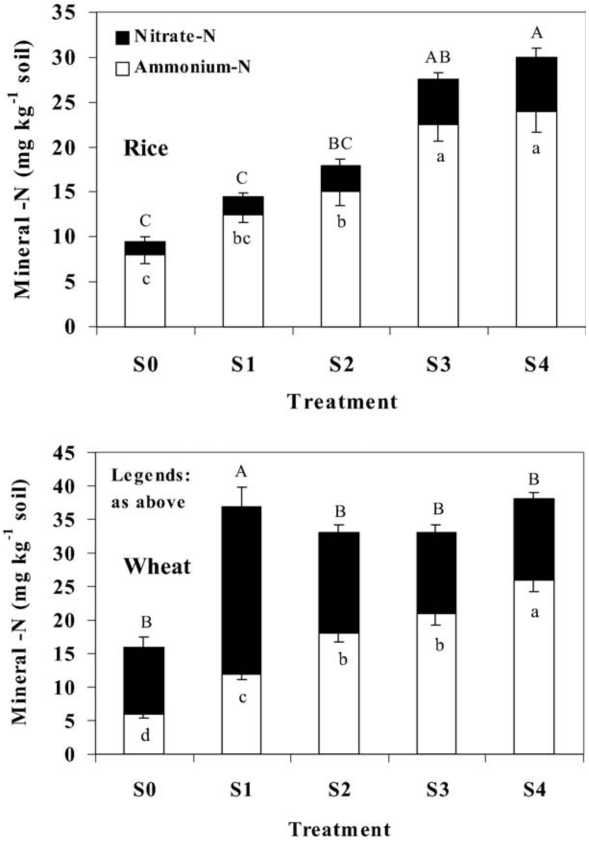

Fig. 1. Mineral-N (NH_4^+ and NO_3^-) in soils during rice and wheat growth at 10 days after top dressing in second year of the crop. Bars above the black portion and down the white portion indicate standard errors (±) of means ($N = 4$). Means not sharing a letter (lowercase for NH_4^+ and uppercase for NO_3^--N) in common differ significantly ($P = 0.05$) from each other according to DMRT. See Table 1 for treatment details.

than in the rest of the treatments (10–15 mg kg^{-1}). In fact all other treatments including the control showed no significant differences ($P < 0.05$ as per DMRT) in their NO_3^--N or total mineral N concentrations (33–38 mg kg^{-1}). However, with respect to NH_4^+-N contents, application of ECC to urea or urea + *S. sesban* (S4) at top dressing had significantly higher values than urea.

3.2. Enzyme activity

The results indicated a strong influence ($P < 0.001$) of treatments to modify dehydrogenase and nitrate reductase activities in soils under rice and wheat crops (Table 2 and Fig. 2). The mean dehydrogenase activity in rice soil at 10 days after top dressing (i.e. 40 days after transplanting) varied from 50.4 mg in control (S0) to 103.2 mg TPF kg^{-1} 24 h^{-1} in urea (S1) treated soil. Significantly ($P < 0.001$) lower values of dehydrogenase activity were measured in soils S3 and S4 due to application of ECC. Similarly, in wheat the dehydrogenase activity was as much as 78 mg TPF kg^{-1} 24 h^{-1} in urea (S1) treated soil and ECC application caused a significant suppression (to 67–68 mg TPF kg^{-1} 24 h^{-1}) in the S3 and S4 treatments. The activity of nitrate reductase followed a similar trend to dehydrogenase activity (Table 2 and Fig. 2). In rice soils, the lowest value of 2.16 mg was measured in the control (S0) and the maximum of 3.6 mg NO_2^--N kg^{-1} 24 h^{-1} in urea treated (S1) soils. The combined application of urea + *S. sesban* + ECC was found to be effective ($P < 0.001$) to reduce the nitrate reductase activity in rice soils. In wheat soil the influence of ECC was also noticed, revealing that treatment S4 (Table 2) had the maximum potential to reduce nitrate reductase activity. The values of dehydrogenase and nitrate reductase activities were found to be linearly and significantly correlated ($R = 0.80$, $P = 0.01$, Pearson's correlation) (Fig. 3).

3.3. Nitrifying organisms

The density of ammonia oxidizing bacteria (AOB) in soil under the rice crop differed significantly ($P < 0.05$) among treatments (Table 2). Comparison of means (DMRT) revealed significantly ($P < 0.01$) higher values in urea alone (MPN 2.25×10^4 g^{-1} dry soil) treatment (S1) than in S3 or S4. The lowest mean MPN value (1.2×10^4 g^{-1} dry soil) of AOB was recorded in treatment S4, where *S. sesban* was included in the top dressing and ECC was applied at both basal and top dres-

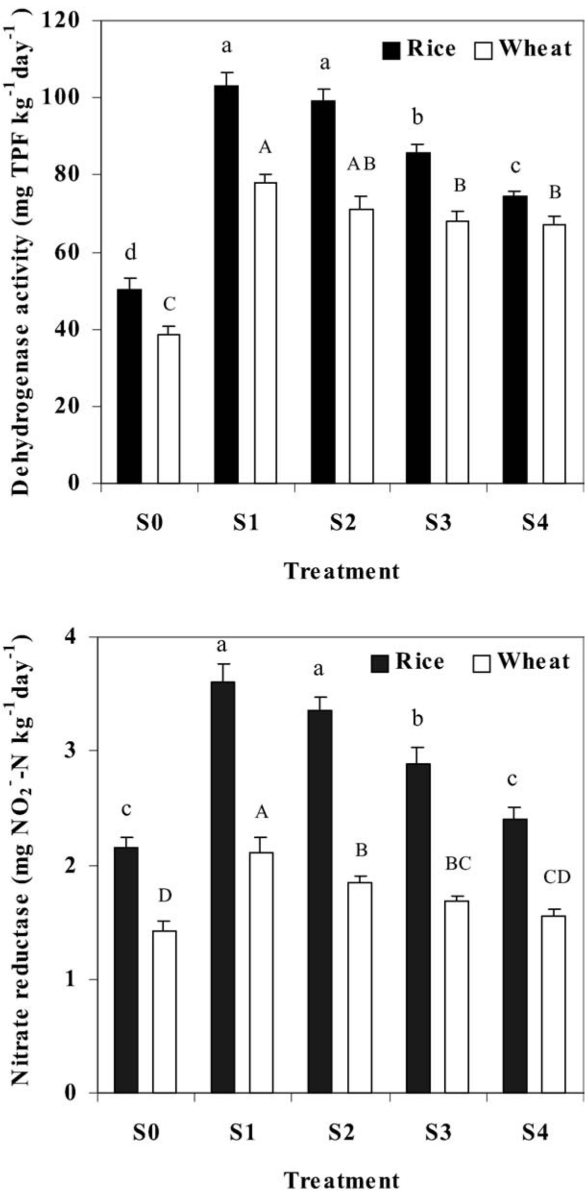

Fig. 2. Dehydrogenase and nitrate reductase activities in soils during rice and wheat growth at 10 days after top dressing in second year of the crop ($N = 4$). Means not sharing a letter in common (lowercase for rice and uppercase for wheat) differ significantly ($P = 0.05$) from each other according to DMRT. See Table 1 for treatment details.

sing. In the case of nitrite oxidizing organisms (NOB), however, no significant differences due to the various treatments were recorded in this study (Table 2 and Fig. 4).

In soils under the wheat crop, populations of AOB varied from 2.8 to 4.1×10^4 g^{-1} dry soil and showed significant differences among treatments. The comparisons of means indicated that treatments S3 and S4

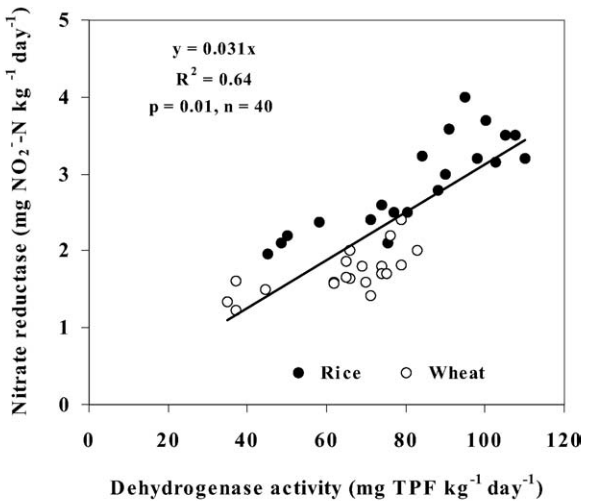

Fig. 3. Effect of green manure (*S. sesban*) and nitrification inhibitor ECC on the relationship between dehydrogenase and nitrate reductase activity in soils under rice and wheat crops at 10 days after top dressing.

were similar but significantly lower than urea alone. The data on NOB in wheat soil samples, however, did not reveal any significant effects of treatments.

4. Discussion

In the present experiment, a rice–wheat cropping sequence was grown for 2 years (1999–2001) on the same soils. Yields and N uptake by rice and wheat were recorded for both the crops [31]. Mineral N (NH_4^+ and NO_3^--N) enzyme activities and concentration of nitrifying organisms (AOB and NOB) in soils were measured only one time in the second year, at 10 days after top dressing (i.e. 40 days after rice transplanting and 35 days after wheat sowing). It is agreeable that more sampling events would have given a better assessment of treatments' effects, but since the 10 days period after top dressing coincides with highly active growth phases of crop (e.g. mid tillering) and most of the applied N generally transformed in soil by that time and susceptible to losses [30], the information obtained in this study has practical relevance in soil fertility and ecological perspectives. For example, similar availability of total mineral N in the urea + *S. sesban* treated soil (S2) and in urea alone (S1) indicates that N in *S. sesban* as a green manure is readily available and can be used effectively by a hybrid rice crop even if applied during crop growth (i.e. top dressing). Benefit of ECC or *S. sesban* + ECC application with urea has been consistently observed by higher total inorganic N contents ($NH_4^+ + NO_3^-$) after N application (*see* S3 and S4 in Fig. 1) in

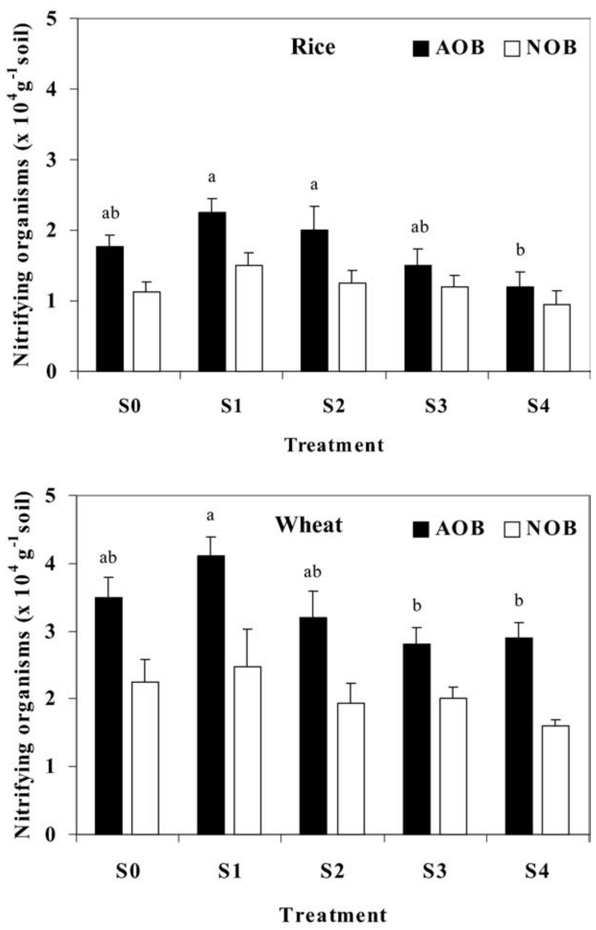

Fig. 4. MPN of ammonia (AOB) and NOB in soils during rice and wheat growth at 10 days after top dressing in second year of the crop ($N = 4$). Means not sharing a letter in common differ significantly ($P = 0.05$) from each other according to DMRT. Treatment differences with respect to NOB were not significant. See Table 1 for treatment details.

soils under rice crop. In case of wheat also application of *S. sesban* in previous rice crop and ECC along with urea have recorded significantly higher NH_4^+-N than urea alone treatment. The higher NH_4^+-N due to *S. sesban* application with urea has been reported due to reducing soil and floodwater pH during decomposition of the leafy materials, which may contribute to lowering the partial pressure of NH_3 and thereby reducing NH_3 losses [13,37] and increased N availability. A combined application of *S. sesban* and ECC in this study has accentuated the availability of NH_4^+-N in the soils due to the effect of *S. sesban* decomposition (as mentioned above) and nitrification retardation by ECC [4,15].

The benefit of *S. sesban* and ECC treatments in this experiment was also reflected in yields and N uptake (i.e. apparent N recovery) by rice and wheat (data not presented). Average N contents at harvest ranged from 7.2 to 8.2 g N kg^{-1} dry matter in rice plants, and 5.3 g N to 9.0 g N kg^{-1} dry matter in wheat plants. Because of treatment effects on N contents and dry matter yields, apparent N recovery varied from 64% (S1) to 75% (S4) in rice (hybrid) and from 50% (S1) to 69% (S4) in wheat [31]. Although in soils under wheat, total mineral N was not significantly different among the treatments (S1–S4), more NH_4^+-N contents in the ECC treatments (Fig. 1) might have played an important role to enhance N availability in soil for a longer time. Whereas, in spite of higher NO_3^- in urea alone (S1) treatment, it had lower N uptake presumably because of less availability of NO_3^--N due to losses through denitrification in subsequent time of wheat growth. Denitrification of NO_3^--N may be induced during wet (irrigation) and dry (post-irrigation) cycles [1]. The benefit of ECC as a nitrification inhibitor has been observed in the fields of irrigated wheat [16], maize [7], cotton [14], and flooded rice [2,5]. Reduced N_2O emission [26] and loss of applied N from 56 to 13% in flooded rice [19] due to application of ECC have also been reported in the past.

Dehydrogenase activity is an indicator and measure for general microbial activity in soil, a factor that might correlate with metabolic activity, microbial biomass or population sizes of specific groups [36]. It has been demonstrated in other studies that this indicator is sensitive to soil management effects [24,27]. In this study, soils receiving applications of urea (S1) and urea + *S. sesban* (S2) had similar dehydrogenase activities but this was greatly reduced by application of ECC (*see* S3 and S4 in Fig. 2). This indicates that apart from nitrification inhibitory property of ECC, it has potential to inhibit the activities of other organisms in soil. It has been documented from other experiments with ECC that it is also effective to reduce the emissions of N_2O, CO_2 and CH_4 from flooded rice soils [8,19]. Since the production of these gases is driven by specific groups of microorganisms, addition of ECC is likely to inhibit their activities and thus emission of gases might be reduced. In our analysis, a strong linear and positive relationship (Pearson's $r = 0.80^{**}$) between dehydrogenase and nitrate reductase activity (Fig. 3) indicates that acetylene slowly released from ECC affects other organisms responsible, for reducing the dehydrogenase and nitrate reductase activities in soils.

For a productive agricultural practice, reduction of nitrate reductase is desirable because it will lead to conserve N by reducing denitrification loss, particularly in flooded rice soils, where it is a major pathway of N

losses [9,30]. Even if such a phenomenon prevails for only a short-term (7–15 days), it would be beneficial to increase N uptakes by growing plants at the basal or top dressing stage. However, the concurrent reduction of dehydrogenase activity due to ECC may not be desirable in soils of the tropical region, which are low in soil fertility. Reduction of dehydrogenase activity may affect transformations of organics and other nutrients in the soil, but this may be a short-term effect, as with nitrification inhibition. Once the supply of C_2H_2 is reduced or stopped the activity is likely to become normal. In a long-term perspective it may prove beneficial because in tropical semi-arid conditions, where weather is harsh because of high temperature and erratic rainfall [33] and soils have low organic matter and N contents, inhibitors like ECC may prove to have potential for protection of added organic matter by retarding its oxidation.

With regard to nitrifying organisms, MPN values obtained in this study only refer to cultivated microorganisms, and generally underestimate the actual bacteria concentration as compared to molecular methods [12,40]. However, they have shown to correlate with soil management regimes on numbers of microorganisms belonging to a functional community [32]. The observed MPN values of ammonia oxidizing bacteria (AOB) from 1.2×10^4 to 4.1×10^4 cells g^{-1} dry soil, and nitrite oxidizing bacteria (NOB) 1×10^4 to 2.5×10^4 cells g^{-1} in soil samples at 10 days were in the range reported by other workers [17,40]. Within 10 days the AOB was increased in urea treated soil, but addition of *S. sesban* or ECC along with urea did not stimulate, rather depressed the AOB populations (Fig. 4). It has been reported that acetylene (C_2H_2), at a partial pressure of 0.1 Pa inhibited the first step of nitrification in soil, i.e. oxidation of NH_4^+ to NO_2^--N by *Nitrosomonas* (ammonia mono-oxygenase), and C_2H_2 at a partial pressure of 10 Pa completely blocked the process [6]. It has been explained that the mode of inhibition is to block the action of the ammonia-oxidizing enzyme, ammonia mono-oxygenase [25]. The inhibitory effect due to C_2H_2 persists for 7 days after acetylene has been removed, and the enzyme is resynthesized by nitrifying bacteria. In contrast to the AOB, no significant effects were seen with the cultivable NOB under rice or wheat, indicating that C_2H_2 might not have any inhibitory effect on the enzymes responsible for oxidizing NO_2^- to NO_3^-.

5. Conclusions

The results of this study demonstrated that combined application of ECC along with urea and *S. sesban* not only improve mineral N availability in soils after top dressing of rice and wheat, but also significantly affect soil enzyme (dehydrogenase and nitrate reductase) activities in favor of N conservation in a semi-arid agroecosystem. This study has also shown that C_2H_2, which slowly released from ECC and inhibits activity of ammonia mono-oxygenase, is associated with reducing population of the AOB. However, since these observations are based on one-time measurements at 10 days after treatments (top dressing), further studies on the temporal variations of biological activities and assessment of N balance will provide a clear pattern of the ecological benefits to be accrued from the adoption of ECC and *S. sesban* along with urea.

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